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(54) Title: IMMUNOTHERAPEUTIC AGENT

(57) Abstract: The present invention relates to mycobacterial extracts comprising lipids, glycolipids and/or carbohydrates and their use in the treatment of allergy.

IMMUNOTHERAPEUTIC AGENT

Field of the Invention

The present invention relates to mycobacterial extracts comprising lipids, glycolipids and/or carbohydrates and their use in the treatment of allergy.

Background of the Invention

Allergies are diseases where the immune response is inappropriately active because of the failure of immunoregulatory networks leading to excessive Th2 lymphocyte-biased responses to allergens. Allergic disorders include asthma, eczema, hayfever, and some forms of food intolerance. These disorders are characterised by the presence of an immune response mediated by Th2 lymphocytes, though some Th1 activity is also present.

Existing allergy therapies concentrate on switching the Th2 cytokine profile towards Th1. Although switching the effector T cell response from Th2 towards Th1 can be beneficial in allergies, this therapy is risky because under some poorly understood circumstances, instead of mutually downregulating each other, the Th1 and Th2 effector cells work together to aggravate the immunopathology. Thus allergen-specific Th1 clones can entirely fail to downregulate a Th2 response (Cottrez *et al.*, 2000; Hansen *et al.*, J Clin Invest 103 175-183 (1999)). Indeed they contributed additional immunopathology, consolidation and macrophage infiltration, despite their ability to downregulate aspects of the Th2 response such as eosinophil infiltration (Hansen *et al.*, 1999).

Similarly in a recent human clinical trial, administration of IL-12 to asthmatics caused some evidence of a switch towards Th1, and some fall in eosinophil count, but failed to decrease the late asthmatic response to allergen exposure (Bryan *et al.*, Lancet 356 2149-2153 (2000)). Moreover a monoclonal antibody to IL-5, a key Th2 cytokine, greatly reduced eosinophil levels in blood and bronchoalveolar lavage, but also failed to affect the late asthmatic response to allergen exposure (Leckie *et al.*, Lancet 356 2144-2148 (2000)).

Regulatory cells that can inhibit the Th2-mediated response to allergen *in vivo* have recently been identified. These cells release IL-10 (Cottrez *et al.*, 2000),

which is already known to specifically decrease IgE production by IL-4-stimulated peripheral blood mononuclear cells *in vitro* (Punnonen *et al.*, J Immunol 151 1280 (1993)). This leads to an alternative approach of therapy by induction of physiological regulatory cells that can control excessive Th2 activity. This approach avoids the potential dangers of unwanted effector activity of Th1 cells (Hansen *et al.*, 1999), and may be very effective if it downregulates *all* Th2 function, rather than merely the levels of a single cytokine.

Mycobacteria are powerful immunological adjuvants. That is to say that when mixed with other antigens they enhance the immune response to these antigens. The immune response that is evoked by mycobacteria is very strongly biased towards a Th1 cytokine profile. This is true whether the organisms are used killed and incorporated into oil (as in Complete Freund's Adjuvant (CFA)) or used alive as in vaccination with Bacillus Calmette Guerin (BCG) or following natural infection with *M. tuberculosis*. This Th1 bias is easily demonstrated using peripheral blood mononuclear cells from normal human donors, which preferentially release Th1 cytokines such as interferon gamma (IFN-γ) in response to mycobacterial antigen, showing that exposure to this genus has primed a Th1 response (Del-Prete *et al.*, J. Clin Invest 88 346-350 (1991)). Mycobacteria do this largely because they trigger release of interleukin 12 (IL-12) which drives the response towards Th1, and they simultaneously impose this Th1 bias on the response to any antigen that is injected with the mycobacterium or emulsified in the CFA (Del-Prete *et al.*, 1991; Romagnani, Immunology Today 13 379-383 (1992)).

Mycobacterium vaccae has particularly potent Th1-adjuvant properties that are manifested even when the organism is used killed, and without incorporation into an oil phase (Abou-Zeid *et al.*, Infect Immun 65 1856-1862 (1997)). It evokes a powerful Th1 response to its own antigens and to antigens associated with it (Abou-Zeid *et al.*, 1997). Similarly if it is injected subcutaneously into the site of previous ovalbumin injections, a killed preparation of this organism can downregulate a pre-existing allergic state in Balb/c mice (Wang & Rook, Immunology 93 307-313 (1998)).

At least some of the ability of micro-organisms to drive Th1-biased responses is attributable to their ability to induce release of proinflammatory cytokines (such as

TNFα) and the pro-Th1 cytokine IL-12 from macrophages, and the Th2-opposing cytokine IFNγ from NK cells (Romagnani, 1992). *Mycobacterium vaccae* is a very potent inducer of IL-12, as expected of an inducer of Th1 lymphocyte responses.

Summary of the Invention

An unexpected effect of lipids, glycolipids and/or carbohydrates derived from mycobacteria has been found. Such lipids, glycolipids and/or carbohydrates have been found to stimulate the development of an allergen-specific cell population in the spleen of subjects to which they have been administered. These cells have been found to be capable of releasing IL-10 and capable of suppressing dysfunctional immune responses in cell transfer experiments. Further, the inventors have found that, surprisingly, there was no increase in IFNγ, indicating that a Th1 response had not been induced. The mycobacterial extract comprising lipids, glycolipids and/or carbohydrates may therefore be used in the treatment and/or prophylaxis of allergic disorders such as asthma, eczema, hayfever, allergic rhinitis or atopic dermatitis.

Accordingly, the present invention provides the use of a mycobacterial extract which extract comprises lipids, glycolipids and/or carbohydrates for the manufacture of a medicament for the treatment of an allergy. Such material may be administered to a subject, for example a subject who suffers from an allergy, in an amount sufficient to relieve the symptoms of the allergy. In a preferred aspect of the invention, the allergy to be treated is selected from asthma, eczema, hayfever, allergic rhinitis, atopic dermatitis, food allergy, allergic reaction to insect bites or to stings, e.g. bee stings, nettle stings or jellyfish stings.

The therapeutic agent of the invention comprises lipids, glycolipids and/or carbohydrates derived from a mycobacterium. In a preferred aspect of the invention the lipids, glycolipids and/or carbohydrates are derived from *Mycobacterium vaccae*. In a further preferred aspect of the invention the extract is substantially free from proteins.

Description of the Figures

Figure 1:

IL-12 release from THP-1 cells induced by culture for 18 hours in the presence of lipid fractions from *Mycobacterium vaccae*.

Figure 2:

A: IL-12 release from normal murine (female BALB/c) spleen cells cultured for 3 days in the presence of lipid fractions from *Mycobacterium vaccae*.

B: IFNγ release from normal murine (female BALB/c) spleen cells cultured for 3 days in the presence of lipid fractions from *Mycobacterium vaccae*.

Figure 3:

A: Protocol used in Example 2.

B: IL-10 release from spleens of mice treated with lipid fractions from *Mycobacterium vaccae* according to the protocol shown in A.

Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The present invention relates to mycobacterial extracts which comprise lipids, glycolipids and/or carbohydrates, and further relates to the ability of the mycobacterial extracts to promote the development of antigen-specific IL-10 secreting regulatory cells.

It has been reported that allergic responses such as asthma and hayfever can be controlled by IL-10 secreting regulatory cells (Cottrez *et al* J Immunol 165 4848-4853 (2000)). The ability of the extracts of the invention to promote the development of such antigen-specific IL-10 secreting regulatory cells indicates that they may be used to treat patients suffering from allergies, in order to control their symptoms.

Extracts of lipids, glycolipids and/or carbohydrates may be obtained from, for example, a frozen mycobacterial cell paste harvested from solid medium or from mycobacteria grown in liquid medium or in a fermentor. Extracts may be prepared by routine methods known to the person skilled in the art. For example,

mycobacteria may be extracted using an organic solvent one or more times to obtain a lipid-containing fraction.

In a preferred aspect of the invention, a mycobacterial extract may be produced by treating the mycobacteria to substantially remove protein content. In a further preferred aspect of the invention, mycobacteria are extracted using a solvent for lipids and/or glycolipids, for example, a non-polar solvent. A suitable non-polar solvent may comprise an organic solvent such as chloroform. In a preferred aspect of the invention the solvent comprises a mixture of chloroform and methanol. In a further preferred aspect of the invention the solvent comprises chloroform and methanol in a ratio of 2:1. Such an extraction will produce a solution fraction with a reduced protein content, and preferably a solution fraction which is substantially free from proteins.

The solid residue from such an extraction may be further refined to extract a carbohydrate component and/or smaller, more polar lipids which were not removed during the earlier extraction. For example, the residue may be resuspended in a solvent, for example in 50% aqueous ethanol. This suspension may be treated to substantially remove protein content. For example, the suspension may be refluxed and the solid, protein-containing material removed. The remaining solution will have a reduced protein content, and preferably, the remaining solution may be substantially free from proteins.

A solution fraction produced from the mycobacteria, and a solution fraction produced from the solid residue may be suitable for use in methods of the present invention. They may be used separately or may be combined.

The combined solutions may be dried by removal of solvent. They may then be further extracted. In a preferred embodiment, they may be extracted in chloroform, methanol and water in a ratio of 10:10:3. The solid residue produced by such a further extraction may be used in the methods of the present invention. The solution fraction of such a further extraction also comprises active material. This solution fraction may be further refined by extraction of undesired material. Undesired material may be extracted by distribution between a polar and non-polar solvent, for example between methanol and petrol. A solution fraction may be further refined by further extractions using organic solvents as described above.

Preferably a solution fraction may be further extracted using chloroform and methanol in a ratio of 2:1. A solution fraction may alternatively or additionally be further treated to precipitate an insoluble fraction, for example by treatment of a solution fraction with acetone.

The activity of a mycobacterial extract may readily be established by one skilled in the art using, for example, a method as described in Figure 3A. Briefly, Balb/c mice are pretreated intraperitoneally with 10μg ovalbumin adsorbed onto alum twice at 0 and 12 days. Such a composition of ovalbumin may be produced by any of the methods well known in the art. They are then treated on day 21 with 5μg of the mycobacterial extract or an equivalent volume of saline. Ovalbumin treatment is repeated at days 42 and 54. On days 61 and 63 ovalbumin is administered at a dose of 50 μg intra-tracheally. On day 65, the spleen is removed and cultured. The cultured spleen is then tested for the ability to produce IL-10 in response to allergen *in vitro*. IL-10 may be assayed by any of the methods well known in the art, for example, IL-10 may be assayed using capture ELISA.

An extract may be from a particular species of mycobacterium, for example *Mycobacterium vaccae*, *Mycobacterium fortuitum*, *Mycobacterium dienhoferei*, *Mycobacterium smegmatis*, *Mycobacterium flavescens* or *Mycobacterium phlei*. The mycobacterium is preferably *Mycobacterium vaccae*. An extract may be from a particular strain of mycobacterium. Particularly preferred strains of *Mycobacterium vaccae* include *M. vaccae* R877R (deposited at the National Collection of Type Cultures Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, United Kingdom on February 13th, 1984 under the number NCTC 11659) and ATCC 15483 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, USA).

Alternatively it may be advantageous and is within the scope of the invention to use lipids, glycolipids and/or carbohydrates derived from more than one species of mycobacterium, or from more than one strain of a mycobacterial species, for example from more than one strain of *Mycobacterium vaccae*.

One aspect of the present invention is the use of the lipids, glycolipids and/or carbohydrates derived from mycobacteria as referred to above in the prevention or treatment of treatment of allergic disorders such as asthma, eczema, hayfever,

allergic rhinitis, atopic dermatitis, food allergy, allergic reaction to insect bites or to stings, particularly to bee stings, nettle stings or jellyfish stings. The lipids, glycolipids and/or carbohydrates may be administered to a patient suffering from or subject to allergy. The treatment may therefore be therapeutic or prophylactic. The condition of a patient suffering from such a disease state can thus be improved. The normal symptoms of a patient subject to an allergy can thus be prevented.

Lipids, glycolipids and/or carbohydrates obtained as outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art. For example, a suitable lipid and/or glycolipid may be put into suspension in, for example, a physiological buffer, isotonic saline or water by physical disruption such as ultrasound. Alternatively it may be put into suspension by ultrasound in the presence of a stable carrier protein, for example lipid-free human serum albumin, to which the lipid and/or glycolipid will bind, providing a stable solution. Alternatively the lipid and/or glycolipid may be formulated as slow release pellets following combination with a suitable carrier molecule, for example cholesterol. A suitable carbohydrate that is linked to a lipid or glycolipid may be formulated in the same way as a lipid and/or glycolipid. A suitable carbohydrate not linked to a lipid or glycolipid may be dissolved in, for example, physiological saline or water for injection. The exact nature of a formulation will depend upon several factors including the particular substance to be administered and the desired route of administration. Suitable types of formulation are fully described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed. 1985, the disclosure of which is included herein of its entirety by way of reference.

The pharmaceutical composition comprising lipids, glycolipids and/or carbohydrates may also contain further ingredients such as adjuvants, preservatives, stabilisers etc. It may further comprise other therapeutic agents. It may be supplied in sterile and pyrogen free form, for example as an injectable liquid; in sterile freeze-dried form which is reconstituted prior to use; or as sterile slow-release pellets. The pharmaceutical composition may be supplied as an isotonic liquid. It may be supplied in unit dosage form.

The substances may be administered by enteral or parenteral routes such as via the oral, buccal, anal or topical route, by subcutaneous, intradermal, intravenous, intramuscular or intradermal injection, by aerosol into the airways, or by other appropriate administration routes. Particularly preferred routes of administration are the oral route or by subcutaneous or intramuscular injection. A physician will be able to determine the required route of administration for any particular patient.

A therapeutically effective amount of a lipid, glycolipid and/or carbohydrate is administered to a patient. The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular patient. Multiple doses may be given. A typical individual dose is from about 0.0001 to 200 mg, preferably from about 0.0005 to 0.5 mg, more preferably from about 0.001 to 0.01 mg, according to the activity of the specific lipid, glycolipid and/or carbohydrate preparation, the age, weight and conditions of the subject to be treated, the type and severity of the allergy and the frequency and route of administration.

The invention will be described with reference to the following Examples, which are intended to be illustrative only and not limiting.

Example 1

Lipids and glycolipids are prominent amongst the microbial components that drive the IL-12 and IFN-γ production, and so bias responses towards Th1 (Almeida *et al.*, EMBO J 19 1476-1485 (2000); Roach *et al.*, Immunology 85 106-113 (1995)). Lipid fractions isolated from *Mycobacterium vaccae* were therefore tested to find out if they share these properties.

Lipids may be prepared from *Mycobacterium vaccae* as follows:

Starting material; frozen mycobacterial cell paste harvested from solid medium or from material grown in liquid medium or in a fermentor.

Lipid extraction. The paste is dried at 60°C +/- 5°C in a vacuum oven for 75 hrs +/- 20hrs. The paste is then extracted with chloroform:methanol 2:1 (v/v) for 90 mins. For example dried organisms derived from 90 gm of paste may be extracted with 240

ml of chloroform:methanol. The solvent extract, comprising lipids and glycolipids, is saved, and the extraction repeated with another 240 ml of chloroform:methanol. The second batch of solvent extract is pooled with the first. The extracted organisms are then air-dried for 20hrs+/-4 hrs. They are then resuspended in 200 ml of 50% aqueous ethanol and refluxed for 2 hrs. This process produces aqueous ethanol solvent which comprises smaller, more polar lipids which were not extracted by the chloroform:methanol, and is substantially free from proteins. This refluxing stage is performed 3 times, and the solvents pooled.

The chloroform:methanol solvent pools and the aqueous ethanol solvent pools may then be combined before drying *in vacuo*, or the two solvent pools may be dried *in vacuo* separately. The fractions used in the experiments reported below were derived from the combined pools as described below.

Fractionation of lipids/glycolipids;

The material is taken up in chloroform/methanol/water 10:10:3.

Fraction 1 – insoluble components, air-dried, and used as it is.

Fraction 2 – soluble components, distributed between petrol and aqueous methanol.

Fraction 3 – petrol layer produced from fraction 2, air-dried and used without further fractionation.

Fraction 4 – aqueous methanol layer produced from fraction 2, dissolve in chloroform methanol (2:1), acetone added to form precipitate.

Fraction 7 – precipitate produced from fraction 4, air-dried

Fraction 8 – soluble components produced from fraction 4, air-dried

For use *in vitro* the fractions are weighed, dissolved, and aliquotted into glass containers and dried again, so that known quantities are present in the containers. Then tissue culture medium or buffered saline is added containing delipidated human or murine serum albumin. Exposure to ultrasonic disintegration then causes the lipids/glycolipids to stay in suspension in association with the albumin.

Fractions were obtained from *Mycobacterium vaccae* as described above. Lipid/glycolipid fractions 7 and 8 were found to drive IL-12 release from THP-1 monocytoid human cell line (Fig 1), and from normal mouse (Balb/c) spleen cells (Fig 2A). The release of IL-12 was assessed by the following method. THP-1 cells were grown up, treated with DMSO for 24 hours in order to differentiate them. The

cells were mixed with the fractions and cultured for 18 hours. The supernatant was then assayed for IL-12 by a capture ELISA method.

Similarly fractions 4 and 7 induced release of IFNγ from normal Balb/c spleen cells (Fig 2B). The release of IFNγ was assessed by the following method. Balb/c spleen cells were taken form the spleens of mice and cultured for 72 hours with the fraction. The supernatant was assayed for IFNγ by a capture ELISA method.

These results suggested that lipid fractions of *Mycobacterium vaccae* would be potent Th1 adjuvants.

Example 2

Since *Mycobacterium vaccae* is a Th1-inducing organism, and since lipid fractions 4, 7 and 8 were potent inducers of the Th1-biasing cytokines IL-12 and IFNγ, it was hypothesised that these lipid materials would act as Th1 adjuvants and induce a Th1 response to the allergen in the Balb/c mouse model of ovalbumin-induced, Th2-mediated allergy. A number of the extracted fractions produced in Example 1 were therefore dried and tested according to the protocol shown in Fig 3A.

Briefly, Balb/c mice were pretreated intraperitoneally with 10μg ovalbumin adsorbed onto alum twice at 0 and 12 days. They were then treated on day 21 with 5μg of the extract or an equivalent volume of saline. Ovalbumin treatment was repeated at days 42 and 54. On days 61 and 63 ovalbumin was administered at a dose of 50 μg intra-tracheally. On day 65, the spleen was removed and cultured. The cultured spleen was then tested for the ability to produce IL-10. IL-10 was assayed using capture ELISA.

The fractions tested were fraction 4, (which contains fractions 7 and 8) and fraction 1. Fraction 3 which had not shown any biological activity *in vitro* was included as an additional control.

Surprisingly, fractions 4 and 1 failed to drive a Th1 response, and instead caused the animals to develop an allergen-specific cell population in the spleen that released IL-10 when cultured *in vitro* in the presence of ovalbumin. There was no release of IL-10 from cultured spleens of any donors if ovalbumin was not added to

the cultures. Therefore the response indicated the presence of allergen-specific IL-10-secreting cells.

Spleen cells from allergic animals that had not been treated with the fractions made no detectable IL-10 in response to ovalbumin (Fig 3B). Moreover there was no increase in IFNγ output in response to ovalbumin, indicating that a Th1 response had not been induced. Similarly the output of IL-5 was not significantly altered, indicating that there was also no increase or decrease in Th2 response. The effects of the lipid fractions were therefore not being mediated by Th1 or Th2. Thus the mode of action of the lipid fractions differs from that of the parent organism which has previously been shown to cause a complete cessation of allergen-induced release of IL-5 (Wang & Rook, 1998). Antigen-specific cells (i.e. T cells) that make interleukin 10 (IL-10) but not IL-5 or IFNγ constitute a population of "regulatory" or "suppressor" T cells (Cavani *et al.*, J Invest Dermatol 114 295-302 (2000); Cottrez *et al.*, 2000; Groux *et al.*, Nature 389 737-742 (1997)). The induction of such cell populations by mycobacterial lipids and glycolipids constitutes a novel finding with multiple uses.

The effect seen with these mycobacterial lipid fractions did not require the lipids to be injected together with the immunogen, or into the same lymphatic drainage area. For example, induction of antigen-specific IL-10-secreting cells was seen in animals immunised by the intraperitoneal route, and treated at an entirely different time point by the subcutaneous route with the lipid fractions alone. This property enables the use of such fractions to promote regulatory cell development even when the immunogen is unknown, as in allergic reaction.

Example 3

Mice (Balb/c females) were immunised by intraperitoneal injection with ovalbumin (10 μg) in alum on days 0 and 12. They were treated with test materials which were Fraction 8 (as prepared in Example 1 above), by the subcutaneous route on days 21 and 35. The mice were reimmunised with the preparation of ovalbumin in alum on days 56 and 68.

On days 76 and 78 the mice were challenged with 0.5 μg of ovalbumin given directly into the trachea. Assessments took place on day 79.

Results

The mice showed a decrease in cellular infiltrate into the lungs, indicative of an anti-inflammatory response. Spleen cells from the treated mice, cultured *in vitro* with anti CD3 for 5 days produced less Interleukin-5 and Interleukin-13, but more Interleukin-10.

Example 4

Subfractions of Fraction 8 were prepared by dissolving Fraction 8 in chloroform and loading onto a silica chromatography column. The fraction was then eluted from the column with methanol dissolved in chloroform, in 10 increasing concentrations of methanol. The concentrations of methanol used were 2%, 5%, 10%, 15%, 20%, 35%, 50%, 75%, 100% and a second 100%. These 10 eluted sub-fractions were then dried down in groups to produce Pool 1 (comprising the 2%, 5% and 10% sub-fractions), Pool 2 (comprising the 15%, 20% and 35% sub-fractions) and Pool 3 (comprising the 50%, 75% and both 100% sub-fractions).

Mice (Balb/c females) were immunised by intraperitoneal injection with ovalbumin (10 µg) in alum on days 0 and 12. They were treated with test materials of Pools 1, 2 and 3 by the subcutaneous route on day 21. The mice were reimmunised with the preparation of ovalbumin in alum on days 42 and 54.

On days 61 and 63 the mice were challenged with 0.5 µg of ovalbumin given directly into the trachea. Assessments took place on day 65.

Results

Mice receiving pools 1 and 3 showed a decrease in cellular infiltrate into the lungs indicative of an anti-inflammatory response.

CLAIMS

1. Use of a mycobacterial extract which extract comprises lipids, glycolipids and/or carbohydrates for the manufacture of a medicament for the treatment of an allergy.

2. The use according to claim 1, wherein the mycobacterial extract comprises lipids and/or glycolipids.

3. The use according to claim 1, wherein the mycobacterial extract comprises carbohydrates.

4. The use according to any one of the preceding claims, wherein the mycobacterial extract is substantially free from proteins.

5. The use according to any one of the preceding claims, wherein the mycobacterial extract is from *Mycobacterium vaccae*

6. The use according to any one of the preceding claims, wherein the allergy is asthma, eczema, hayfever, allergic rhinitis, atopic dermatitis, food allergy or allergic reaction to insect bites or to stings.

7. A method of treating an allergy which comprises administering to a subject suffering from or subject to allergy a mycobacterial extract which extract comprises lipids, glycolipids and/or carbohydrates in an amount sufficient to prevent, control or alleviate the symptoms of the allergy.

8. A method according to claim 7, wherein the mycobacterial extract comprises lipids and/or glycolipids.

9. A method according to claim 7, wherein the mycobacterial extract comprises carbohydrates.

10. A method according to claim 7, wherein the mycobacterial extract is substantially free from proteins.

11. A method according to claim 7, wherein the mycobacterial extract is from *Mycobacterium vaccae*.

12. A method according to claim 7, wherein the allergy is selected from the group consisting of asthma, eczema, hayfever, allergic rhinitis, atopic dermatitis, food allergy or allergic reaction to insect bites or to stings.

13. An extract comprising lipids, glycolipids and/or carbohydrates which extract is derived from *Mycobacterium vaccae* and which is substantially free from proteins.

14. A pharmaceutical composition comprising lipids, glycolipids and/or carbohydrates derived from *Mycobacterium vaccae* for use in the therapy of an allergy.

15. A pharmaceutical composition according to claim 14, which is sterile and pyrogen free.

16. A pharmaceutical composition according to claim 14 or 15, which is isotonic.

17. A pharmaceutical composition according to any one of claims 14 to 16, in unit dosage form.

18. A method substantially as hereinbefore described in any one of the examples.

Figure 1

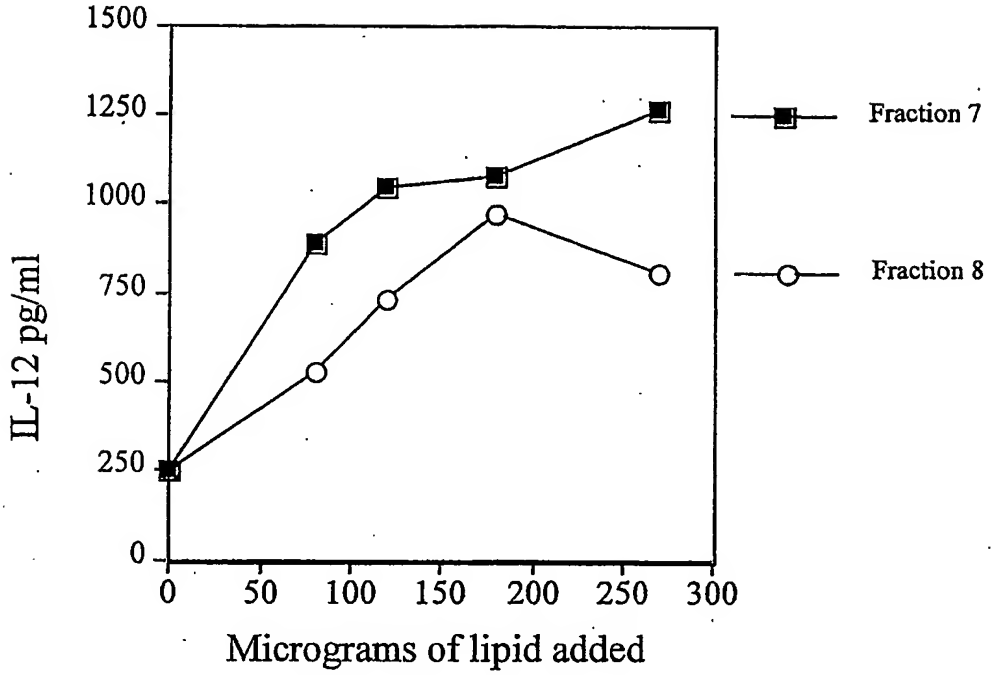
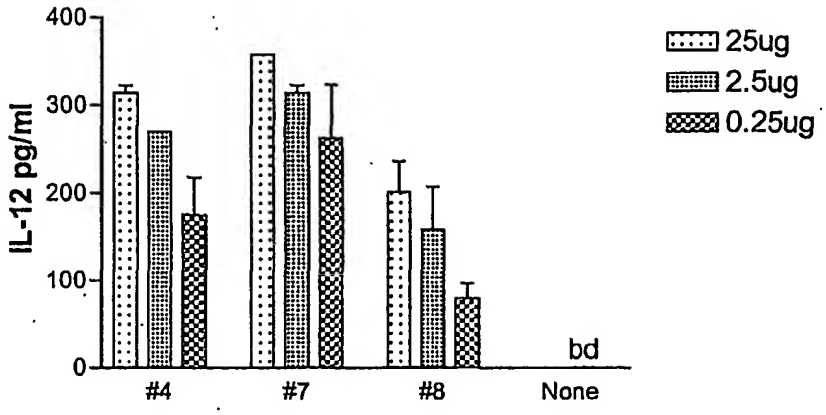

1500
1250
1000
750
500
250
0
IL-12 pg/ml
0
50
100
150
200
250
300
Micrograms of lipid added
Fraction 7
Fraction 8

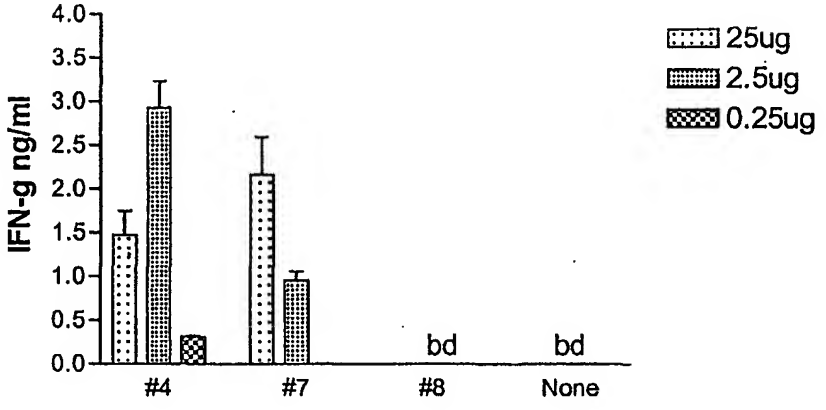
Figure 2

A



Lipid fraction added to normal mouse spleen cells

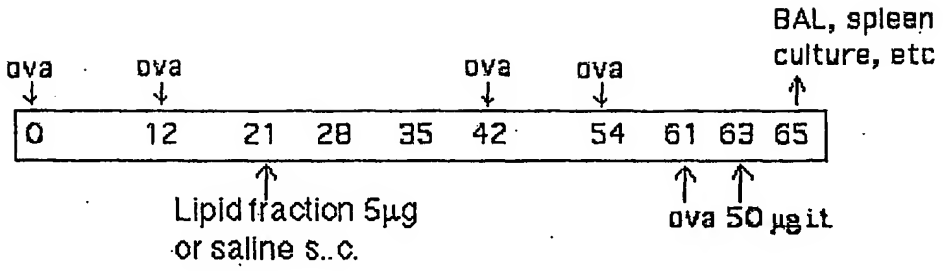
B



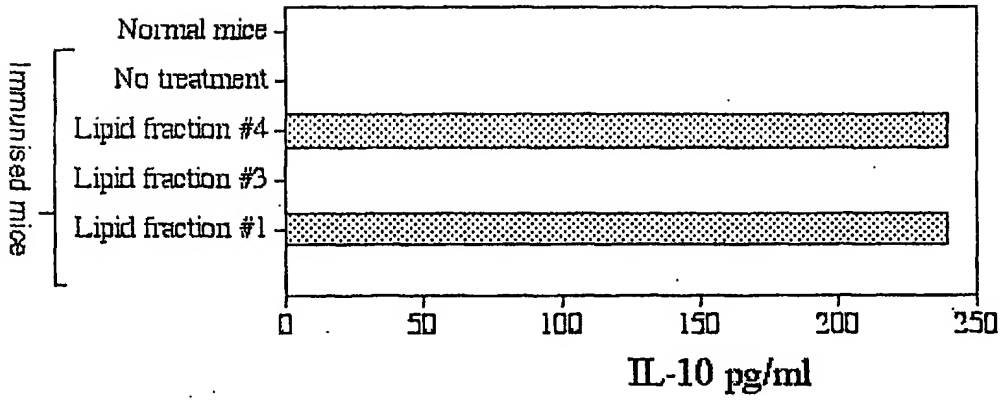
Lipid fraction added to normal mouse spleen cells

Figure 3

A


BAL, spleen culture, etc
ova
ova
ova
ova
0
12
21
28
35
42
54
61
63
65
Lipid fraction 5µg or saline s..c.
ova 50 µg i.t

B


Normal mice
No treatment
Lipid fraction #4
Lipid fraction #3
Lipid fraction #1
Immunised mice
0
50
100
150
200
250
IL-10 pg/ml